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Term: ((antifreeze or (anti ADJ freeze)) NEAR3 (protein or peptide or polypeptide)) and (ice ADJ (cream or confection))

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L2: Entry 2 of 2 File: USPT Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096867 A
TITLE: Frozen food product

ASNM:
Good Humor-Breyers Ice Cream, Division of Conopco, Inc.

ASZZ:
Good Humor-Breyers Ice Cream, Division of Conopco, Inc.

ABPL:
Plant anti freeze proteins can advantageously be incorporated into frozen confectionery products, provided they have the capability of limiting the growth of ice crystals

BSPR:
Antifreeze proteins have been described in the literature, see for example Marilyn Griffith and K. Vanya Ewart in Biotechnology Advances, Vol 13, No 3, pp 375-402, 1995. Antifreeze proteins generally possess one or more of the following properties: thermal hysteresis, inhibition of ice recrystallisation, control of ice crystal shape and interaction with ice nucleators.

BSPR:
For example AFPs have been suggested for enhancing the cryopreservation of biological materials (WO 91/12718, Agouron Pharmaceuticals, WO 91/10361, The Regents of the University of California). Also AFPs have been suggested to prevent leakage from liposomes e.g. in cosmetic or pharmaceuticals (see WO 96/20695). A further possible application is to increase the freezing tolerance of plants by including therein (or transgenetically producing therein) an AFP (See J. Cell. Biochem. Suppl. vol. 14e, 1990, page 303 XP002030248, Lee et al, abstract R228). Also fish AFPs have been suggested for use in food products for example in frozen yoghurt or ice cream (U.S. Pat. No. 5,620,732 Pillsbury and WO 96/11586, HSC Research and development limited partnership).

BSPR:
A number of literature places have suggested that AFPs may potentially be used for favourably influencing the textural properties of frozen confectionery products such as ice cream. However most of these documents do not provide a teaching how these favourable properties can actually be achieved in practice.

BSPR:
WO 96/11586 teaches the application of fish antifreeze polypeptides in frozen fermented food products. This document does not teach the use of specific AFPs derived from plants in these products.

BSPR:
WO 96/39878 describes the application of AFP in ice-cream. Suitable AFPs for this application may be derived from blood and muscle tissue of antartic fish, artic fish, worms and insects. Again no teaching is provided that plant AFP can be used.

BSPR:
WO 92/22581 describes a plurality of polypeptides derived from the extracellular spaces of winter rye. Several possible applications of these polypeptides are described in general, among these is ice-cream. However no teaching is provided which of the polypeptides should be selected to obtain a good quality ice-cream.

Applicants have found that only specific proteins from winter rye are suitable for use in ice-cream (see examples).

BSPR:
Applicants have found that a great number of plants which up till now have not been listed as such, contain a significant level of AFPs. On the other hand it has been found that not all AFP containing plants provide the right type of AFP for favourably influencing the texture of frozen confectionery. Applicants now aim at providing a particular novel selection of plant sources which surprisingly provide on the one hand good AFP properties and on the other hand are capable of favourably influencing the textural properties of ice cream.

BSPR:
AFPs obtainable from the above mentioned sources can be used in any suitable frozen confectionery product. For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet, sorbet, ice milk and frozen custard, water-ices, granitas and frozen fruit purees. For some purposes the use in fermented frozen food products is less preferred.

DEPR:
A liquid pre-mix for preparing ice-cream was made by mixing:

DEPR:
The mixes can be used in the preparation of a ice-cream by whipping with a conventional house-hold mixer to an overrun of about 100%, followed by quiescently freezing into a house-hold freezer.

DEPR:
A liquid premix for the preparation of ice-cream was prepared by mixing:

DEPR:
The mix can be used for the preparation of ice-cream by whipping it with a conventional house-hold mixer to an overrun of about 70% followed by freezing under quiescent conditions in a house-hold freezer. After two months storage the composition according to the invention had a markedly better texture than the control sample.

DEPR:
Carrot root anti-freeze proteins were purified by gel permeation chromatography as follows:

DEPR:
20 .mu.l aliquots of sample were applied to a SMART superdex 75 column (Pharmacia) pre-equilibrated in 50 mM Tris/HCl pH7.4 containing 0.15M NaCl (Buffer E) at a flow rate of 40 .mu.l/min and components separated by gel permeation at the same flow rate in equilibration buffer. The eluate was monitored at OD 280 and OD 215. 80 .mu.l fractions were collected between 0.85 and 0.89 ml, 40 .mu.l fractions between 0.89 and 1.24 ml and 100 .mu.l fractions between 1.24 and 3.0 ml. The void volume (Vo) of the column was 0.91 ml as determined by the retention volume of a solution of Blue Dextran. The superdex column was calibrated by application of 10 .mu.l of a solution containing 5 mg/ml BSA (Mr 66 kDa, retention (Ve)=1.02 ml), 3 mg/ml Carbonic anhydrase (Mr 29 kDa, Ve=1.22 ml), 2 mg/ml Cytochrome C (Mr 12.4 kDa, Ve=1.41 ml) and 2 mg/ml Aprotinin (Mr 6.5 kDa, Ve=1.59 ml) and a standard curve plotted of Ve/Vo against log Mr. Fractions containing anti-freeze activity were identified by the spat assays in Example I, with an activity peak that showed a retention volume of 1.16 ml and an apparent molecular weight of 40 kDa. These measurement confirmed that the 38 kDa band from cold acclimatised carrots was an anti-freeze peptide.

DEPR:
Cell cultures for producing anti-freeze proteins were made as follows:

DEPR:
Root extract from cold acclimatised carrot roots was prepared by scrubbing freshly pulled cold acclimatised carrots in cold water. The tops are removed and the juice extracted employing a domestic juice extractor (Russell Hobbs, model no 9915). The juice was frozen in 1 liter blocks and stored a -20.degree. C. prior to collection for use in ice cream trials.

DEPR:
The carrot AFP juice was added to the following ice cream formulation

DEPR:
Ice-cream was prepared by freezing above formulation and aeration to 106% overrun.

DEPR:
Samples were equilibrated at -18 C. in a Prolan Environmental cabinet for approximately 12 hours. Three samples were chosen representatively from each batch of ice cream and a slide was prepared from each in a Cryostat temperature control cabinet by smearing a thin layer of ice cream from the centre of each block onto a microscopic slide. A single drop of white spirit was applied to the slide and a cover slip was then applied. Each slide, in turn, was then transferred to a temperature controlled microscope stage (Leit LaborLux S, Leica x10 objective, temperature -18.degree. C.). Images of ice-crystals (about 400 individual ice-crystals) were collected and relayed through a video camera (Sanyo CCD) to an image storage and analysis system (LEICA Q520MC).

DEPR:
The stored ice crystal images were highlighted manually by drawing around the perimeter which then highlights the whole crystal. Images of the highlighted crystals were then measured using the image analysis software which counts the number of pixels required to complete the longest straight line (length), shortest straight line (breadth), the aspect ratio (length/breadth). The data for each individual ice crystal of a batch of ice cream was imported into a spreadsheet where analysis of the data set was carried out to find the mean, and standard deviation.

DEPR:
The ice Cream Hardness Measurements were carried out using a Hounsfield H10KM Universal Tester, a Hounsfield 100N Load Cell and a 10 cm Cylindrical Stainless steel probe. The ice-cream samples were prepared by 16 Hour incubation of 486 ml ice cream blocks in a Prolan Temperature Control Cabinet set at -18.degree. C.

DEPR:
The ice cream block was removed from Prolan temperature control cabinet and placed the Hounsfield H10KM Universal Tester. The 10 cm cylindrical probe was pushed into the ice cream block at a constant rate of 400 mm/min to a depth of 20 mm. The maximum force recorded during the compression was used and expressed as the ice cream Hardness. If cracking or brittle fracture of the sample was observed this was indicated in the right hand column

DEPV:
a) Initial ice crystal size is smaller in ice cream containing Carrot AFP, thus carrot AFP is inhibiting recrystallization inhibition.

DEPV:
b) Ice crystals in carrot AFP ice cream are retarded in their recrystallization processes.

DEPV:
c) Ice crystal shape in carrot AFP ice creams are not significantly different from crystal shapes seen in conventional ice creams.

DEPV:
d) Material properties of ice cream containing carrot AFP are modified from those noted for conventional ice cream. Namely, ice creams are harder than conventional ice cream but still softer than ice-cream containing e.g. fish AFPs. Secondly, ice cream containing carrot AFP was observed to fracture.

DETL:

Material	Properties Mean Aspect Ratio	Mean Crystal Length/ Breadth	Ice Crystal Size Parameters Mean Crystal Factor/ Fracture	Brittle Crystal Ratio/ N	Mean Crystal Hardness/ tion	Crystal Shape observa-	Sample	Mean um -	Mean um -
Carrot	26.79 .+-. 1.43	19.00 .+-. 1.15 .+-. 40.8	Yes	AFP -	1.3	0.9	0.013	0.024	fresh
Carrot	33.48 .+-. 1.13	24.61 .+-. 1.37 .+-. 59.9	Yes	AFP -	1.3	0.9	0.013	0.020	Abused
Cont.	-	33.67							

.+-. 24.79 .+-. 1.12 .+-. 1.38 .+-. 27.3 No Fresh 1.1 0.8 0.008 0.018 Cont. -
61.7 .+-. 46.54 .+-. 1.11 .+-. 1.37 .+-. 32.7 No Abused 2.7 2.0 0.010 0.020